

AMERICAN MUSEUM *Novitates*

PUBLISHED BY THE AMERICAN MUSEUM OF NATURAL HISTORY
CENTRAL PARK WEST AT 79TH STREET, NEW YORK, N.Y. 10024

Number 3144, 21 pp., 5 figures, 4 tables

August 14, 1995

Discovery of the Costa Rican Poison Frog *Dendrobates granuliferus* in Sympatry with *Dendrobates pumilio*, and Comments on Taxonomic Use of Skin Alkaloids

CHARLES W. MYERS,¹ JOHN W. DALY,²
H. MARTIN GARRAFFO,³ ANTHONY WISNIESKI,⁴
AND JOHN F. COVER, JR.⁵

ABSTRACT

Dendrobates granuliferus, previously thought to be a characteristic endemic of Pacific-side rain forest in the Golfo Dulce region, was found in sympatry with *Dendrobates pumilio* on the Caribbean coast of southeastern Costa Rica, near the Panamanian border. The sympatric frogs were easily separated by features of coloration and skin texture. Relative abundance in microsympatry was about 100 *pumilio*:4 *granuliferus*.

Inasmuch as *Dendrobates pumilio* is sometimes strikingly polymorphic within populations, the initial identifications were tested with bioacoustical, skin-alkaloid, and allozyme data. These com-

parisons negate the possibility of intrapopulation polymorphism and are consistent with the determination of the rare species as *D. granuliferus*. Previous inferences that *D. granuliferus* and *D. pumilio* are sister species are neither supported nor repudiated by present data.

Interpopulational and even individual variation in the skin toxins of these species is extraordinary and probably reflect dietary differences as well as genetic factors. Current knowledge of the dendrobatid alkaloids is briefly reviewed in a systematic context. With a few exceptions, skin chemistry has not been useful in supporting taxonomic differ-

¹ Chairman and Curator, Department of Herpetology and Ichthyology, American Museum of Natural History.

² Research Associate in Herpetology, American Museum of Natural History; Chief, Laboratory of Bioorganic Chemistry, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health.

³ Visiting Associate, Laboratory of Bioorganic Chemistry, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health.

⁴ Curator of Reptiles and Amphibians, Baltimore Zoo.

⁵ Curator of Rain Forest Exhibits, National Aquarium in Baltimore.

entiation of closely related species. But underlying genetic mechanisms for alkaloid sequestering (and synthesis?) support the monophyly of a suprageneric group of aposematic dendrobatids (tropical poison frogs). Within this group, the monophyly of *Phylllobates* (true dart-poison frogs) and of *Phyl-*

lobates + *Dendrobates* is supported by alkaloid data. The monophyly of *Minyobates* (dwarf poison frogs) also is corroborated, although in this case the alkaloid character is one of loss and especially in need of further study.

INTRODUCTION

The Costa Rican poison frog *Dendrobates granuliferus*⁶ was discovered by Edward H. Taylor in 1952, in the Pacific lowlands adjacent to the Golfo Dulce of southern Costa Rica (Taylor, 1958). One of Taylor's collecting companions was Paul Allen, who was then working on his now-classic *The Rain Forests of Golfo Dulce* (Allen, 1956). Allen's work drew attention to the isolation of these magnificent forests, whose biota is related to that of the Atlantic lowlands; the Golfo Dulce region shares many species with the Atlantic rain forests, but there is conspicuous endemism as well, of which *Dendrobates granuliferus* has been considered a good example.

From the beginning (Taylor, 1958), *Dendrobates granuliferus* has been compared with *D. pumilio* of the Atlantic versant and they have come to be regarded essentially as sister species. In a review of Central American dendrobatids, Savage (1968: 774) observed that,

D. granuliferus appears to be a close ally of, and replaces *D. pumilio* of the Atlantic versant, in the Golfo Dulce area (fig. 13). Essentially, the Golfo Dulce area supports a dendrobatid fauna corresponding to the fauna of Atlantic lowland Costa Rica, but completely isolated from it by the Talamanca-Chiriquí range and the Pacific lowland dry forest zones to the north and south.

More recently, Savage (1982: 521) listed *D. granuliferus* and *D. pumilio* among several "species pairs" presumed to have been separated by uplift of the now-intervening mountains. Silverstone (1975: 38), however, had questioned whether speciation had been completed:

D. pumilio is closely related to an allopatric species, *D. granuliferus*, with which it probably was geographically and genetically continuous before the onset of orogeny and aridity in Costa Rica. The similarity of

a specimen from Sixaola, in the Caribbean drainage of Costa Rica (BM 1956.1.6.54), to *D. granuliferus* calls into question the specific distinctness of *D. pumilio* from *D. granuliferus*.

The discovery therefore of a *granuliferus*-like frog on the Atlantic side of lower Central America—in sympatry with a population of *Dendrobates pumilio*—is of interest in several respects and is the subject of this paper.

GEOGRAPHY AND FIELD OBSERVATIONS

The location of sympatry is in southern Limón Province, Costa Rica, at 9°38'08"N, 82°49'21"W—as plotted on topographic sheet 3644 IV (Amubri), 1:50,000, Instituto Geográfico Nacional, San José, Costa Rica, 1969. The local place name, as shown on this map, is "Río Sand Box"⁷. Collections were made in forest on either side of the Río Sand Box a short distance upstream (northwest) from the road to the nearby settlement of Bribri. There were remnants of lowland rain forest at an elevation of approximately 200 m above sea level.

The site was visited on June 22–25, 1989, by Daly and Wisnieski, who found a population of red-orange (usually) to orange smooth-skinned frogs assignable to *Dendrobates pumilio* (fig. 1, top). On June 24, two granular-skinned frogs, whose coloration was immediately reminiscent of *Dendrobates granuliferus* (fig. 1, bottom), were found about 3 m apart at the forest edge. These two frogs were preserved on February 21, 1990, after being maintained for photography and recording at the National Aquarium in Baltimore.

⁷ Until the last few decades, English (especially the Jamaican variety) was more prevalent than Spanish in the Bocas del Toro region of Panama and adjacent Costa Rica, leading to quaint bastardizations of many place names. The cartographer's pen has preserved this instance of it.

⁶ We are aware that the name *granuliferus* was recently corrected to "*granulifer*." The change seems unwarranted; see Nomenclatural Note at end of this paper.

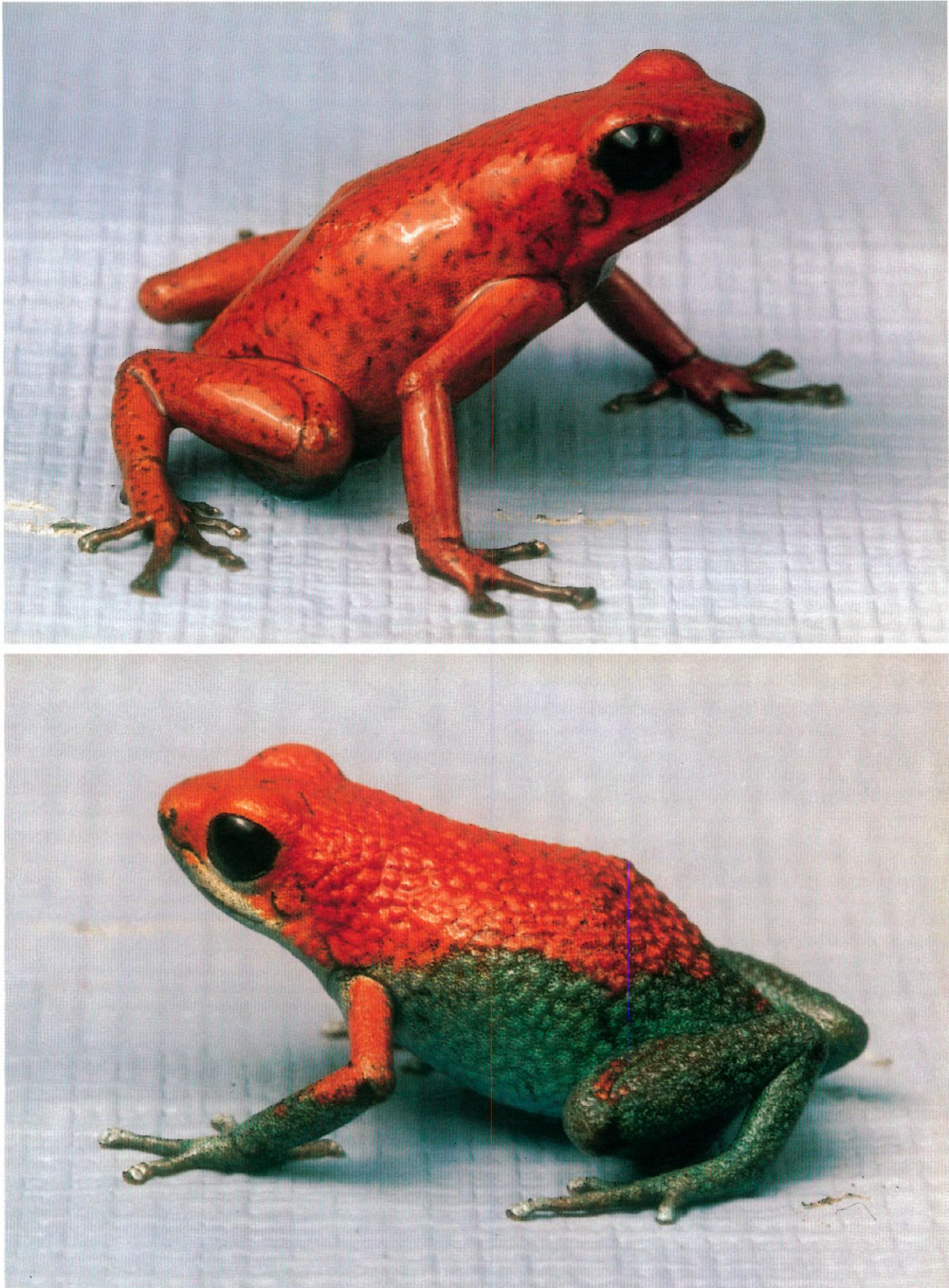


Fig. 1. Two species of *Dendrobates* occurring together in lowland rain forest on the Atlantic versant of Costa Rica. **Top:** *Dendrobates pumilio* O. Schmidt, a species confined to the Atlantic versant from Nicaragua to western Panama, where it becomes explosively polymorphic in coloration and other features. **Bottom:** *Dendrobates granuliferus* Taylor, formerly known only from the Golfo Dulce region on the Pacific side of Costa Rica (this specimen is preserved as a museum voucher in the series AMNH A-134011–134012).

A second collection was obtained a year later during a full day of intensive collecting, on June 16, 1990, by Cover, Daly, and Wisnieski, in company of Charles Nishihira. These collectors found an additional four specimens identified as *Dendrobates granuliferus*, along with numerous *D. pumilio*. Two of the *granuliferus* were preserved and the other two were transferred alive to the American Museum, where skins were taken for study of defensive secretions and the carcasses frozen for allozyme electrophoresis and other analyses.

The four *Dendrobates granuliferus* from 1990 were in the same section of forest—north of the Río Sand Box—that yielded the first two individuals in 1989. Specimens of *D. pumilio* were found as close as 1 m to specimens of *D. granuliferus* and so the two are microsympatric. Unlike *D. granuliferus*, however, *D. pumilio* was found throughout the forest, both north and south of the Río Sand Box. Very low population density and simple sampling error could well account for not detecting *granuliferus* in forest on the southern side of the Río Sand Box, although species of the *histrionicus* group often occur in demes that seem not to occupy all available habitat.

100 *D. pumilio* were collected in the Río Sand Box forest during the one day in 1990, in addition to the four *D. granuliferus*. Inasmuch as *granuliferus* seems to be behaviorally as conspicuous as the frogs in the sympatric population of *pumilio*, the collectors' ratio of over 100 *pumilio* to 4 *granuliferus* reflects a very substantial difference in relative abundance in the Río Sand Box forest (where all *granuliferus* seen were caught but many *pumilio* were not).

The Río Sand Box site is fairly remote and it seems highly unlikely that *Dendrobates granuliferus* was introduced from the Pacific coast by human agency. Additionally, a British Museum specimen collected some 30 years earlier, purportedly in the Sixaola area, also seems referable to *D. granuliferus*, thus providing independent indication that the occurrence is natural. The British Museum specimen was listed under *D. pumilio* by Silverstone (1975), who thought that it "is nearly as granular as *D. granuliferus*" (p. 37) and that it "calls into question the specific distinctness" (p. 38) of *pumilio* and *granuliferus*.

"Sixaola," if applied to the settlement of that name, lies on the lower Río Sixaola, which forms the border between Costa Rica and Panama. The Río Sand Box locality is farther inland, roughly 27 km NW of Sixaola and some 3 km N of the Río Sixaola.

Museum Specimens: *Dendrobates granuliferus*, BMNH 1956.1.6.54 (collected about 1955–1956, by G. M. Gillott, United Fruit Co.), Sixaola, Talamanca, 800 ft, [Limón Prov.], Costa Rica [specimen not examined, identification based on comments in Silverstone, 1975: 37–38]; AMNH A-134011–134012 (June 24, 1989), 134013–134014 (June 16, 1990), from Río Sand Box (*vide supra*). *Dendrobates pumilio*, AMNH A-134019–134046 (June 22–25, 1989, and June 16, 1990), *idem*.

MORPHOLOGY AND COLORATION

The common *Dendrobates* at the Río Sand Box site is a relatively smooth-skinned frog that is overall red-orange (occasional individuals being orange), with inconspicuous gray flecking (fig. 1, top). It fits well among the mostly red populations of *Dendrobates pumilio* in southeastern Costa Rica (Daly and Myers, 1967, note 3) as well as some in the explosively polymorphic populations of adjacent Panama (e.g., see color plate in Myers and Daly, 1983). In the preserved sample, dorsal skin texture grades from smooth (most individuals) to weakly granular, in keeping with Silverstone's (1975: 37) description.

The other, rare kind of frog in the Río Sand Box forest differs from the above in having strongly granular skin and a blue-green color on the flanks, hindquarters, lower arms and venter (fig. 1, bottom). It closely resembles *Dendrobates granuliferus* from the region of the type locality in southwestern Costa Rica. Good descriptions of this species were given by Savage (1968) and Silverstone (1975).⁸

Soft-bodied animals cannot be measured precisely, but error was minimized by taking measurements carefully with dial calipers at

⁸ Unfortunately some of the colors were wrongly reproduced in Silverstone's (op. cit.) frontispiece II. As confirmed by Silverstone (letter to Myers, January 12, 1976), the "limbs of *D. granuliferus* should be blue-green, not light blue [and] the stripes of *D. auratus* should be green [not whitish]."

one sitting. The sympatric samples differ slightly in body size, as shown by the following comparisons of snout-vent length (SVL in mm) of adult frogs:

Dendrobates granuliferus

2 ♀ 21.1–21.3, \bar{x} = 21.20

2 ♂ 20.2–20.5, \bar{x} = 20.35

Dendrobates pumilio

19 ♀ 21.3–23.9, \bar{x} = 22.67 \pm 0.157, *s.d.* = 0.684

6 ♂ 21.1–22.0, \bar{x} = 21.62 \pm 0.151, *s.d.* = 0.371

Interspecific differences between the means by sex are significant at the 0.01 level, even for the small samples of males (for males, t = -4.437; females, t = -2.964). Measurements for the smaller kind of frog fit within the 19–22 mm range reported for *D. granuliferus* by Siverstone (1975: 9), who gave means of 20.8 mm for adult females and 20.7 mm for males. The larger frogs fit within the upper part of Silverstone's (loc. cit.) size range for *D. pumilio*, in which there is considerable interpopulational variation in head-body length (unpublished data).

VOCALIZATION

Vocalizations of specimens from the first (1989) collection were recorded at the National Aquarium in Baltimore. The tape recording was played through a real-time Kay 7851 spectrograph, and waveforms and spectrograms were printed on continuous paper from a Honeywell LS-8 Linescan Recorder run at 10 mm/sec. Call lengths then were measured to the nearest 1 mm (= 0.1 sec) and number of notes counted for each call; isolated notes or small clusters of notes (< 5) were excluded, as were a few obviously interrupted or unusually broken calls. Note length and frequency in notes throughout selected calls were measured using a Kay 5500 DSP Sona-Graph, which was used for production of sound spectrograms (figs. 2–4).

Chirp Calls: The advertisement calls of the sympatric frogs are trains of notes that have the harsh, nonmusical (and nonbuzzlike) auditory quality of "chirp calls," a class of vocalizations considered synapomorphic for the *histrionicus* group of *Dendrobates* (Myers et al., 1984). Within this class, however, the calls of different species may be fundamen-

tally dissimilar in average call length, note repetition and pulse rate, note length, and frequency, as indicated, for example, in the small sample of recordings of captive *D. pumilio* and *D. granuliferus* from the Río Sand Box (table 1, figs. 2–4).

Note Repetition Rate and Pulsation: The call of *Dendrobates pumilio* from Río Sand Box is given relatively quickly at a rate of about 6 notes/sec vs a much slower rate of about 2–3 notes/sec for sympatric *D. granuliferus*. The individual notes are rapidly and rather uniformly pulsed, as seen in the waveforms of figures 2 and 3.

Note Length: Average note duration is a short 0.07 sec in the sample of *D. pumilio* calls, compared with 0.18 sec in the longer and randomly variable *granuliferus* notes. *D. pumilio* often starts with a sequence of especially short notes (\approx 0.03–0.05 sec), which then become uniformly longer (\approx 0.07–0.08 sec) and constant throughout the rest of the call. Thus, if initial notes were excluded, average note duration would be higher than the 0.0678 sec calculated for *D. pumilio* in table 1; the difference (0.066 sec) between shortest and longest notes in table 1 is in fact the result of sampling 10 beginning and 10 middle notes within a single *pumilio* call.

As indicated above, *D. granuliferus* has much longer notes than *D. pumilio*, and the *granuliferus* notes vary relatively randomly throughout the calls. Durations of the four sequential notes shown in the wide-band spectrogram of a *granuliferus* call (fig. 3) are, from left to right, 228, 216, 197, and 197 msec; the next five notes (not shown) are 184, 200, 194, 191, and 225 msec. This is a 14.5 sec call comprising 27 notes in a range of 0.082–0.241 sec (\bar{x} = 0.2042 \pm 0.0186 sec), including four scattered pairs of same-length notes which are preceded and followed by either shorter or longer notes. Such variable duration of individual notes within the same sequence is unusual among dendrobatid chirp calls and probably is autapomorphic in *D. granuliferus*.

Frequency: Calls of Río Sand Box frogs differ also in emphasized frequency and degree of frequency modulation. The greatest energy output falls within 3–4 kHz in a sample of four *D. pumilio* calls, which show the following intercall variation: 3000–3900, 3200–3900, 3300–3900, and (in fig. 4A) 3400–

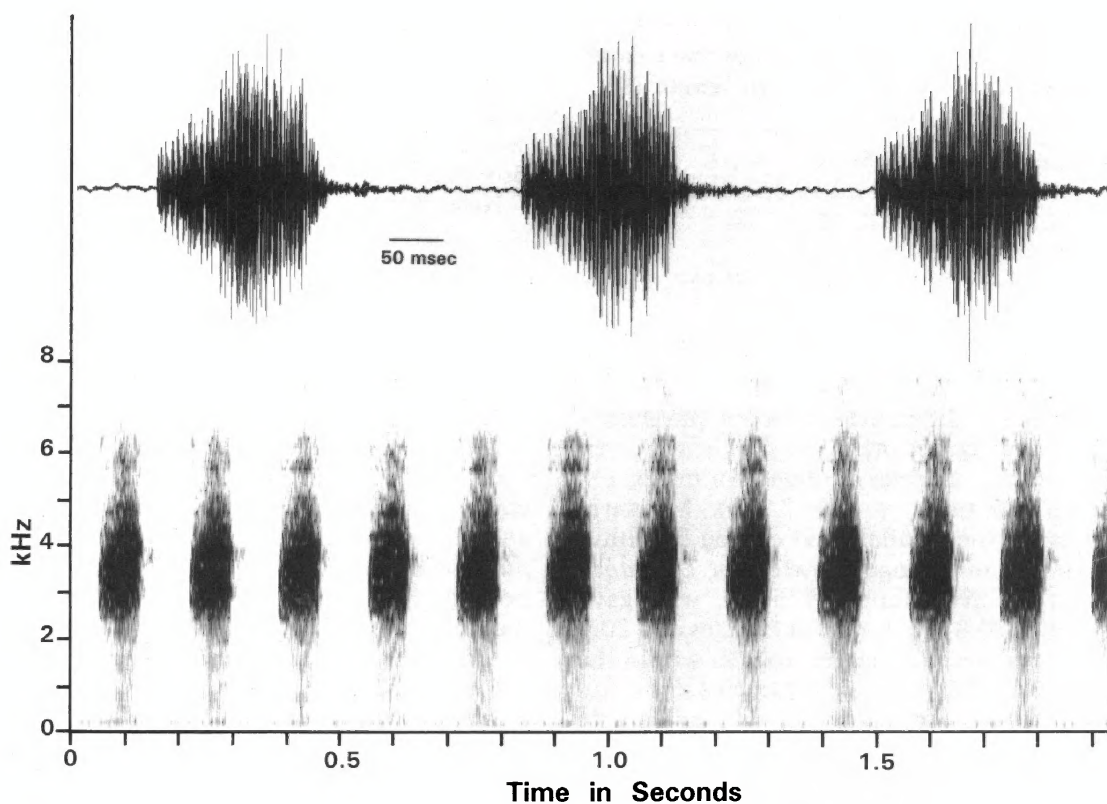


Fig. 2. Chirp call of *Dendrobates pumilio* from Río Sand Box, near Bribri, Costa Rica, graphed with wide-band, 300 Hz filter. Below, sound spectrogram from middle of a 25.8 sec call. Above, expanded waveforms of first three notes in spectrogram. Recorded in terrarium at National Aquarium in Baltimore, at temperature of 21°C (AMNH herpetology reel 262).

4000 Hz. There is a weak tendency for two emphasized harmonics within the emphasized range (fig. 4A). The first and last of the four calls above have relatively constant frequency throughout, whereas the first several notes of the other two calls start about 100–200 Hz lower before rising to the emphasized frequency that is held through the rest of the note and the rest of the call. Frequency modulation in these calls therefore is minimal.

In contrast, in *D. granuliferus* the peak emphasized frequency is much higher and the notes are frequency modulated throughout the calls. Maximum energy output is in the broad range of 3.4–4.6 kHz. Video scrolling through power spectra (not illus.) of computer-stored signals shows the frequency to be lowest in the early part of each *granuliferus* note, then shifting up to above 3.9 kHz throughout the greatest part of each note.

The sound spectrograms here assigned to *D. granuliferus* compare reasonably well with a published description and spectrogram of Pacific-side *granuliferus* (Myers and Daly, 1976: 234–235, fig. 20), including variable-length notes and emphasized frequency slightly above 4 kHz (op. cit., fig. 22F). The call of Río Sand Box *D. pumilio* resembles in most respects the call of *pumilio* throughout its range (Myers and Daly, 1976: Myers et al., 1984, fig. 10C), except that frequency seems geographically variable in this species. In various populations of *D. pumilio*⁹ in west-

⁹ The “eastern group” of populations discovered by Myers and tentatively assigned to *D. pumilio* in Myers and Daly (1976) are here excluded. They belong to an unnamed species that ranges from the Atlantic coast to the continental divide and which seems most closely related to *D. arboreus* of western Panama (Myers, in prep.).

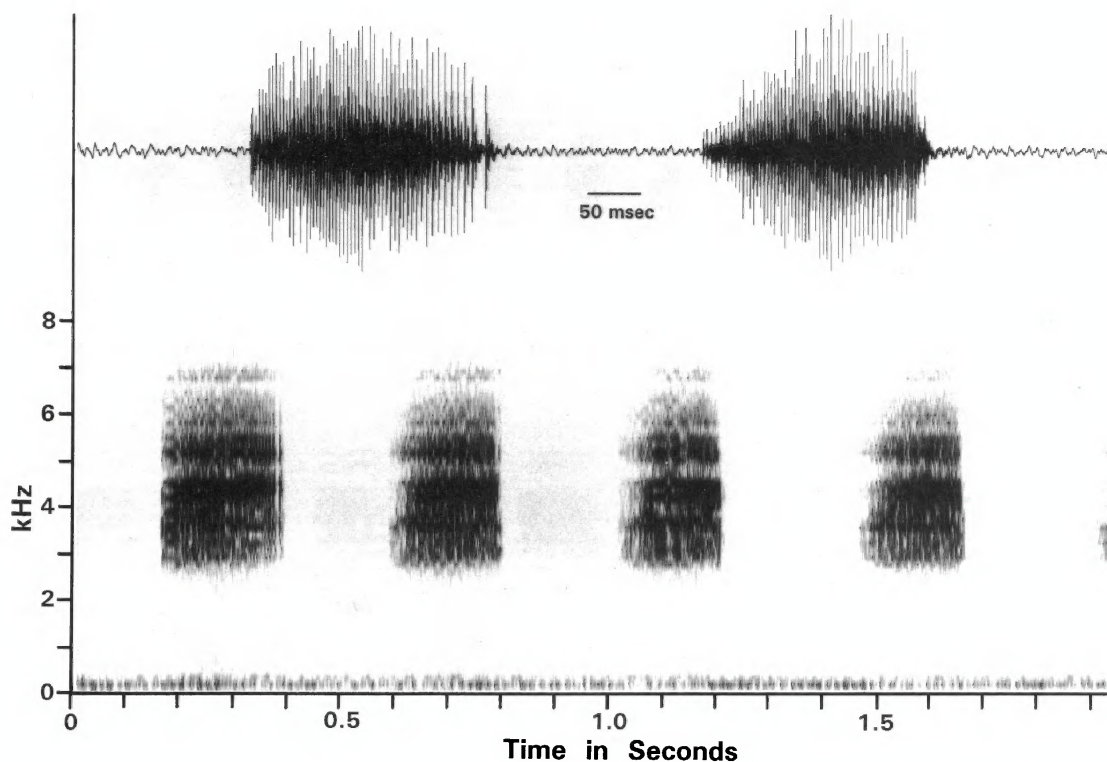


Fig. 3. Chirp call of *Dendrobates granuliferus* collected in sympatry with *D. pumilio* at Río Sand Box, near Bribri (wide-band, 300 Hz). Below, sound spectrogram from middle of a 14.5 sec call. Above, expanded waveforms of first two notes in spectrogram. Recorded in terrarium at National Aquarium in Baltimore, at temperature of 21°C (AMNH herpetology reel 262).

ern Panama, the emphasized frequency is above 4 kHz—as in *D. granuliferus*—whereas most energy output is between 3–4 kHz in Río San Box *pumilio* and in another, much more northern Costa Rican specimen (Myers and Daly, 1976: 234, fig. 19). See Conclusions for speculation on this observation. In any case, differences in call between the Río Sand Box populations are clearly indicative of separate species and are consistent with their assignments to *Dendrobates pumilio* and *D. granuliferus*.

Postscript: A recently published study (Meyer, 1993) of Pacific-side *Dendrobates granuliferus* came to our attention as the present paper was going to press. It includes a comparison of calls of several species of the *Dendrobates histrionicus* group, but, unfortunately, the presentation is not reliable.

One sound spectrogram (Meyer's fig. 9) for *D. pumilio* contains a power spectrum in

which the emphasized (dominant) frequency is appreciably lower than the region of greatest energy output in the accompanying notes and is in no way representative of them. A spectrogram (Meyer's fig. 11) attributed to *D. speciosus* shows a dominant frequency about 1 kHz too high for that species and cannot be reconciled with the frequency data in Meyer's table 1 (for sound spectrograms of bona fide *D. speciosus*, see Myers et al., 1984, fig. 10B, and Jungfer, 1985, fig. 3). The signals analyzed by Meyer appear to be well-recorded (not over-loaded) or else well-filtered, but his tabulated Frequenzbereiche (frequency-range of the whole signal) is determined to some degree by machine variables and therefore is not a reliable character. The frequency range mistakenly cited for *D. arboreus* in Meyer's table 1 actually represents intercall variation in the dominant frequency peak, which originally was described as "usually at

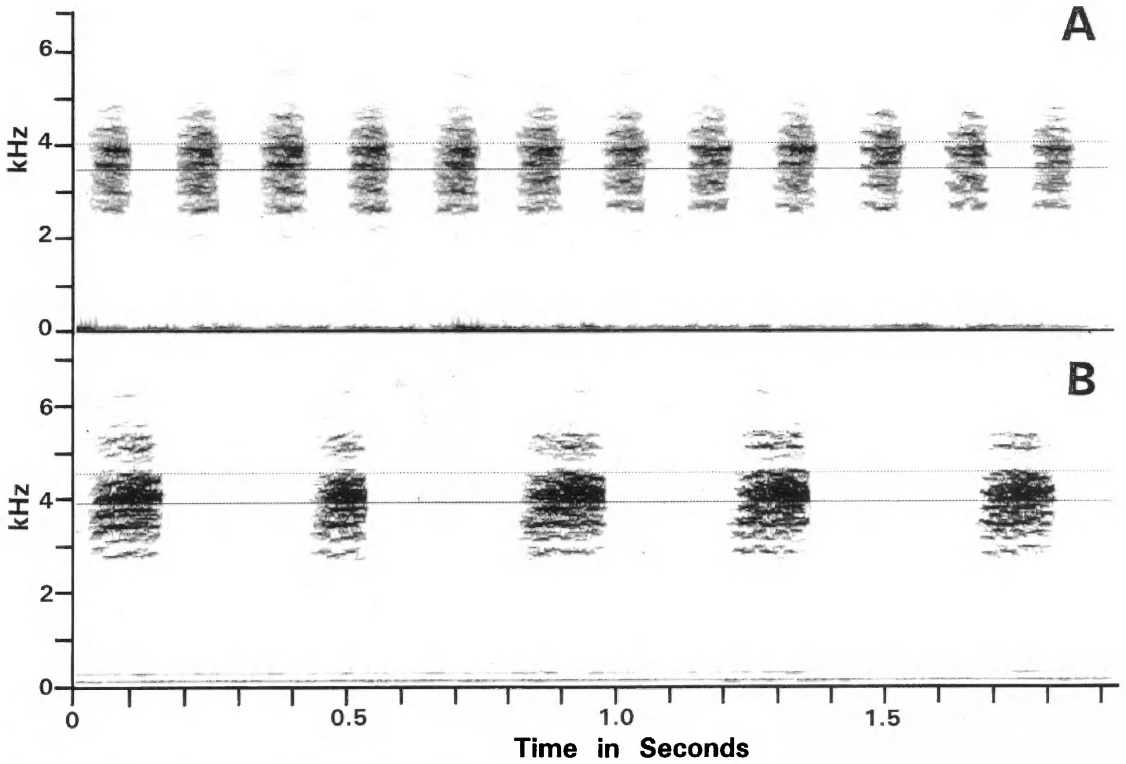


Fig. 4. Narrow-band (59 Hz) sound spectrograms, middle of (A) 8.5 sec call of *Dendrobates pumilio* and (B) 7.0 sec call of sympatric *Dendrobates granuliferus*. As shown by paired cursors, greatest energy output is in the range 3440–4000 Hz in *D. pumilio* and 3920–4560 Hz in *D. granuliferus*. Both recorded at National Aquarium in Baltimore at 21°C (AMNH herpetology reel 262).

about 3800 Hz in a total range of about 3700–4000 Hz” (Myers et al., 1984: 18).

During interactions with other frogs, dendrobatids commonly emit sounds that differ in various ways from their advertisement calls. Meyer described for *D. granuliferus* a

courtship call comprised of exceptionally long (about 0.5 sec) notes given with noticeably rising frequency (see complete note in his fig. 8). For note duration in the advertisement call, he gave an average of 0.213 sec with a standard error of 0.0086 sec (*N* = 23, range

TABLE 1
Analysis of Vocalizations of Captive *Dendrobates granuliferus* and *D. pumilio* Collected in Sympatry^a

Species	Temp. (°C)	N (calls)	Call Length (sec) Mean ± 1 SD (Range)	Notes per sec Mean ± 1 SD (Range)	N (notes)	Note length (sec) Mean ± 1 SD (Range)
<i>D. granuliferus</i>	20	17	9.76 ± 10.61 (2.0–39.0)	1.99 ± 0.33 (1.5–2.6)	—	—
<i>D. granuliferus</i>	21	4	6.20 ± 6.08 (1.6–14.5)	2.59 ± 0.57 (1.9–3.1)	54 ^b	0.1786 ± 0.0340 (0.093–0.241)
<i>D. pumilio</i>	21	11	14.60 ± 8.37 (5.3–32.2)	6.19 ± 0.16 (5.9–6.5)	80 ^c	0.0678 ± 0.0132 (0.025–0.091)

^a Recorded in terraria at National Aquarium in Baltimore. Number of frogs contributing calls is not known.

^b All notes in four calls (5, 5, 17, 27 notes per call).

^c Sample of 20 notes in each of four calls (first 10 notes + 10 consecutive notes from middle of call).

not given). Meyer does not mention the sort of random variation in note duration of *D. granuliferus* that is discussed in the present paper; but the two notes shown from an advertisement call (his fig. 7) seem to differ by about 12 msec.

ALLOZYME ELECTROPHORESIS

Material and Methods: Two specimens from each of the following three populations were used for starch-gel allozyme electrophoresis.

- A. Pacific versant of Costa Rica, about 6 km E Palmar Norte, Puntarenas Province (*Dendrobates granuliferus* from region of type locality).
- B. Atlantic versant of Costa Rica, Río Sand Box near Bribri, Limón Province (disjunct population of *Dendrobates granuliferus*, sympatric with "C" below).
- C. Same locality as "B" above (sympatric *Dendrobates pumilio*).

These samples fortuitously became available before completion of the laboratory part of a much larger electrophoretic study of the *Dendrobates histrionicus* group generally and the *D. pumilio* complex specifically (Escalante et al., MS.). So, except for the small sample sizes, the present analysis will be compatible with the larger study.

A total of 23 enzymatic loci were assayed according to standard procedures (to be detailed in Escalante et al., MS.) and analyzed with *Biosys-1* (Swofford and Selander, 1989).

Results: Of the 23 loci examined, four loci were invariable within and among these three populations (see table 2). Six of the remaining 19 loci were suggestive of fixed differences between *D. granuliferus* and *D. pumilio* (ADA, EST, GOT1, GPD1, MPI, and PGM), whereas others showed frequency differences that were significant using a contingency chi-square test (ADH, LDH2, PEPA, and PEPC, $P < 0.05$).

In comparing the Pacific- and Atlantic-versant samples of *D. granuliferus* against one another, 14 of 23 loci were invariable (table 2). Although allele compositions differ at nine loci, only the two possibly fixed differences (at ADH and G3PD) are significant at the 0.05 level. Wright's $F_{ST} = 0.461$ for the two *granuliferus* populations.

Using genetic distance calculated over variable loci, the two samples assigned to *D. granuliferus* are closer to each other than they are to *D. pumilio* (table 3). Although the Atlantic-side population of *D. granuliferus* appears less differentiated from sympatric *D. pumilio* than is the allopatric Pacific-side population, these data are inconclusive. Allozyme differences appear consistent with the interpretation of separate populations (species) of *Dendrobates* at the Río Sand Box.

SKIN TOXINS

GAS CHROMATOGRAPHIC PROFILES AND IDENTIFICATION OF ALKALOIDS

Analyses were conducted of alkaloids in extracts of individual skins and of pooled samples of *Dendrobates granuliferus* from populations on the Pacific and Atlantic versants and of the microsympatric population of *Dendrobates pumilio*. Gas chromatographic traces for most samples are shown in figure 5. Analysis by gas chromatography-mass spectrometry and gas chromatography-Fourier transform infrared spectroscopy led to identification of most alkaloids present in these skin extracts. Their occurrence among the various extracts is shown in table 4.

COMPARISON OF POOLED SAMPLES

Gas chromatographic traces for pooled skins from Pacific-side *Dendrobates granuliferus* (Palmar Norte, 4 skins) and for the Atlantic-side *Dendrobates pumilio* (Río Sand Box, 30 skins) are shown on the right side of figure 5 (samples 2C and 4C). Unfortunately, a pooled skin sample for the Atlantic-side *Dendrobates granuliferus* was not obtained because only two skins (male and female) were available for analysis. The pooled samples are useful in adding further trace compounds, perhaps existing at levels too low for detection in individual skins. As an indication of the extraordinary variation among individuals, however, a female *granuliferus* contained trace amounts of an alkaloid (237D) not detected in the pooled samples of four other skins. Although a total of 31 alkaloids (including isomers) were detected in the 30-skin *pumilio* sample, the two individual skins add an additional four compounds (221G*, 231C from the male, 207J from the female,

TABLE 2
Allele Frequencies of *Dendrobates granuliferus* from Pacific Versant (Population A) and Atlantic Versant (B) of Costa Rica, and of *Dendrobates pumilio* (C, Sympatric with B)

Population				Population			
Locus	A	B	C	Locus	A	B	C
ADA				IDH1			
B			.50	B		.50	
C			.50	D	1.00	.25	.75
E	1.00	1.00		H			.25
ADH				I		.25	
D		1.00	.50	IDH2			
E	1.00			D	.50	1.00	1.00
F			.50	E	.50		
EST				LDH1			
D	1.00	1.00		C	1.00	.75	1.00
F			1.00	D		.25	
ESTD				LDH2			
B	.50	.50	1.00	C	1.00	1.00	.25
E	.50	.50		D			.75
FUM				MDH2			
C	1.00	1.00	.50	A			.25
D			.50	C	1.00	1.00	.25
GDH				F			.50
C		.25		MPI			
D		.50		D	1.00	.75	
E	1.00	.25	1.00	F		.25	
GOT1				I			.75
D			1.00	J			.25
E	1.00	1.00		PEPA			
GPD1				D	1.00	1.00	.25
C			1.00	E			.75
D	1.00	1.00		PEPC			
GPD2				D		.25	1.00
C			.50	E	1.00	.75	
D	1.00	1.00	.50	PGM			
G3PD				D			.50
F		1.00*	1.00	E			.50
G	1.00			F	1.00	1.00	

N = 2 frogs each population, except N = 1 at asterisk (G3PD).
Invariable loci: CK, F16P, GOT2, MDH1.

and 219B from both the male and female—see table 4).

COMPARISONS OF INDIVIDUAL SKINS

The gas chromatographic traces demonstrate marked differences between individual

frogs taken from the same population—in figure 5, compare the single skin profiles (A and B) from each of three populations of *Dendrobates granuliferus* and one of *D. pumilio*. Although several of these comparisons are of male/female pairs, data are inadequate to test whether the differences are sexually correlat-

ed (sexual dimorphism) or uncorrelated (true individual variation). Therefore we use "individual variation" in the loose sense.

An especially striking instance of such variation is shown by two *D. granuliferus* from the vicinity of Puerto Jiménez on the Península de Osa of Pacific Costa Rica (compare samples 1A and 1B in fig. 5 and table 4). Sample 1A contains major amounts of both 19-carbon histrionicotoxins, whereas sample 1B has instead 15- and 17-carbon histrionicotoxins. The former contains the 19-carbon decahydroquinolines, while the other has 15- and 17-carbon decahydroquinolines.

GENERAL COMPARISONS OF PROMINENT ALKALOIDS

All but one of the *D. granuliferus* and *D. pumilio* extracts contain as major and minor alkaloids a group of 19-carbon decahydroquinolines (mol. wts. 269 and 271) and a group of 19-carbon histrionicotoxins (mol. wts. 283, 285, 287). This set of alkaloids often occurs together in dendrobatid frogs, although it is completely lacking in some species (and in some Panamanian populations of *D. pumilio*) and, in others, the histrionicotoxins are major and the decahydroquinolines are virtually absent. But, oddly, in the present extracts, these alkaloids are all lacking in a single *granuliferus* skin (table 4, sample 1B), which contained 15- and 17-carbon compounds instead (see above under *Comparisons of Individual Skins*). Histrionicotoxins are known only in nature from anuran skin.

Such extreme differences between frogs caught at the same time and place have not heretofore been documented for any dendrobatid, although long-term sampling of *D. pumilio* on Isla Bastimentos in Panama has indicated that changes in alkaloid profiles can occur over time (unpubl. data, see Daly et al., 1993: 251). Present data suggest that temporal change may similarly occur in *D. granuliferus* at Palmar Norte. The 1990–1992 Palmar Norte extracts (2A–2C) are compared in table 4 with data from a 9-skin sample collected by Daly and Myers at approximately the same locality a quarter of a century previously. This early sample, which had been studied by much less sensitive analytical

TABLE 3
Matrix of Genetic Distance Coefficients: Nei's (1978) Unbiased Distance Above Diagonal, Modified Rogers' Distance (Wright, 1978: 91) Below Diagonal

Population	A	B	C
A. Pacific <i>D. granuliferus</i>	—	.138	.698
B. Atlantic <i>D. granuliferus</i>	.380	—	.567
C. Sympatric <i>D. pumilio</i>	.682	.628	—

techniques (Daly et al., 1978), afforded among the major alkaloids two decahydroquinolines (219A, 243A) that are absent in the recent samples. The minor alkaloids from the earlier study included two histrionicotoxins (259A, 291A), an allopumiliotoxin (341A), and a few trace compounds not detected in the present study. Thus, the profile in the 1967 sample is clearly different from the 1990–1992 samples from virtually the same locality. Yet, a 5-skin 1988 sample from the same general locality is very similar to the 1967 sample, with 16 (73%) of 22 alkaloids shared.

The pumiliotoxin-A class (pumiliotoxins and allopumiliotoxins) is well represented in *Dendrobates granuliferus*, with at least one minor or major compound of this class being present in all samples, whereas only trace amounts of one pumiliotoxin and one allopumiliotoxin were detected in the Río Sand Box *D. pumilio*. Allopumiliotoxin 267A is either a major or minor alkaloid in all the *granuliferus* samples, but was not detected in the *pumilio* extracts. Pumiliotoxin-A class compounds are known only in nature from anuran skin.

Other classes of dendrobatid alkaloids seem only randomly represented as major alkaloids in the present samples. Examples: Isomers of the pyrrolizidine 223H occur in most, albeit not all, of the samples but it is a major alkaloid only in the single male *pumilio*; one of these isomers also is reported from a myrmicine ant (Jones et al., 1980). Several 3,5-disubstituted indolizidines occur only as minor or trace alkaloids in some of the *granuliferus* and *pumilio* extracts. Various 5,8-disubstituted indolizidines were detected in all but one extract, usually as only minor or trace components except for 205A and 223A, which were major alkaloids in one *granuliferus* and

TABLE 4
Skin Alkaloids from Several Populations of *Dendrobates granuliferus* and a Sympatric Population of *Dendrobates pumilio*

Alkaloids ^a	<i>Dendrobates granuliferus</i>									<i>Dendrobates pumilio</i>		
	Puerto Jiménez		Palmar Norte					Rio Sand Box		Rio Sand Box		
	1A 1 skin	1B 1 skin	2A 1 skin ♂	2B 1 skin ♀	2C 4 skins	--- 5 skins	--- 9 skins	3A 1 skin ♂	3B 1 skin ♀	4A 1 skin ♂	4B 1 skin ♀	4C 30 skins
	Jan. 87	Jan. 87	June 90	June 90	June 92	Dec. 88	July 67	June 90	June 90	June 90	June 90	June 90
Histronicotoxins												
235A		++				+	+					
259A		++				+	+					
283A	+++		+++	++	+++	++	+++	++	++	+++	+++	+++
285A	+++		+++	++	+	+	+++	++	++	+++	+++	+++
285C	+++		++	+	++	++	++	++	++	++	+++	++
287A	+		++	+	+			++	++	++	++	++
291A							++					
Pumiliotoxins												
251D		++		+	+							
277B			+	+	+							
307A					++	++	++		+			
307B					++	+	++					
323A			++	+	++	++	+++	+	+			+
Allopumiliotoxins	+++	+++	++	++	++	++	+++	++	++			+
267A					+							
323B					+	+	++					
341A												
357					+							
Decahydroquinolines												
195A									+			+
211A						++	++					+
219A		+++				+++	++					
233F			++	++	+++	+++	++	+	++	+		+
243F		++				+++	++					
243A			++	++	+++	+++	++		++	+		+
269AB	++		+	+	++	+	+	++	+	+	+	++
269A	++		++	++	++	+	+	+++	+++	++	+	++
269B					+			+				
271C*			+	+	+			+	++		+	+
271D*			++	++	+						+	+
275B			+		+			+	+			
3,5-Pyrrolizidines			+++	+++	+++			+++	+++	+++	+++	+++
223H (H',H'') ^b			++	++	++			++	++	++	++	++
251K			+		++							
3,5-Indolizidines										++		++
195B									+			
223AB				+	++			+	++	+		+
275C												
5,8-Indolizidines	+++	+			+			+	+			
205A					+			++	+			
207A		+			+			++	+			
223A ^c			+		+	+		++	++	+++	++	++
231C										+		
237D				+								
245D*					+							
251N					++				+			
1,4-Quinolizidines		+										
209E									+	+	+	+
219B	+											
231B									+	+	+	+
249C									++	++	++	++
259C			++	+	+			+	++	++	++	++
261B*									+			+
263A					+							+
275A								+		+		++
277A												+
Pyrrolizidines												
183B												+
197B	+	+++		+++	+	++	++	++	++			++
225C					+			+	++			
225H*												
Piperidines												
213*									++			
225B									+			
239E*												+
Tricyclics ^d												
207J											+	
209G					+							
Unknowns												
201*	++	+	++	++	+	+		+				
207E												
207G*									+	++		+
207H*										+		++
221G*										++		
221H*												
223L*									++			
267J*										+	++	++
281B							+					+
309B												

^a For structures see Daly et al. (1993); for distribution in dendrobatid frogs see Daly et al. (1987). The amounts (+++ = major, ++ = minor, + = trace) follow the notation used in prior publications. Two or more entries separated by commas indicate two or more isomers in their order of elution from the gas chromatograph.

one *pumilio* extract, respectively. Various 1,4-disubstituted quinolizidines occur as minor or trace alkaloids in most extracts but are especially well represented in the Atlantic versant *granuliferus* and sympatric *pumilio* samples. The pyrrolidine **197B**, which occurs as a major alkaloid in two individual *granuliferus* skins and as a minor or trace alkaloid in several other extracts, also is known from certain myrmicine ants (Jones et al., 1982). Piperidines were detected only in Atlantic versant frogs as minor (*granuliferus*) or trace (*pumilio*) alkaloids.

ATLANTIC VS. PACIFIC POPULATIONS OF *D. GRANULIFERUS*

The pair of Atlantic versant *D. granuliferus* share 25 (66%) of their combined 38 alkaloids with one or more population samples of Pacific lowland *granuliferus*. If comparisons are made only with the Palmar Norte sample, there are 24 (63%) compounds shared. Six minor alkaloids (quinolizidine **249C**, pyrrolidine **225C**, piperidines **213***, **225B**, and unknowns **221H***, **223L***) and seven trace alkaloids are not shared with any of the Pacific-side *granuliferus* samples.

SYMPATRIC *D. GRANULIFERUS* AND *D. PUMILIO* COMPARED

The two Atlantic-side *granuliferus* share 22 of their 38 alkaloids (58%) with sympatric *pumilio*—about the same degree of difference when compared with a single Pacific-side population of *granuliferus* (see above). Seven alkaloids (18%) from these two frogs are unique in the sense of being found neither in the Pacific-side *granuliferus* nor in sympatric *pumilio*, which itself has nine (26%) of its 35

alkaloids not detected in any of the *granuliferus* samples. The difference between sympatric *granuliferus* and *pumilio* therefore seems meaningful.

However, attention also is drawn to a special similarity between these populations: Six trace or minor alkaloids (decahydroquinoline **195A**, quinolizidines **219B**, **249C**, **263A**, and unknowns **207G***, **207H***) shared by the sympatric populations of *pumilio* and *granuliferus* were not detected in any other sample of *granuliferus*. But despite this special resemblance and the sizeable number of shared compounds in general, comparison of “unique” alkaloids in the paragraph above suggests that the two Atlantic-side *granuliferus* skins were drawn from a population with an overall different set of skin toxins than possessed by sympatric *pumilio*. It is not known, however, whether the difference in skin toxins in this case reflects a subtle difference in diet or feeding behavior or a genetic difference in ability to uptake or biosynthesize specific alkaloids. Nonetheless, the extraordinary variation in skin toxins of *Dendrobates pumilio* and *D. granuliferus* now seems likely to reflect differences in dietary uptake of many alkaloids. This statement is made in the context of recent discoveries, as reviewed below.

A BRIEF REVIEW OF THE SYSTEMATIC SIGNIFICANCE OF DENDROBATID SKIN TOXINS

Several decades of field and laboratory work, the latter conducted with ever increasing analytical sophistication, have resulted in the characterization of nearly 300 skin alkaloids from several dozen species of mostly

← Two alkaloids, **223F** and **197B**, were identified as minor constituents of the July 1967 Palmar Norte sample on reexamination of the mass spectral data. Additional trace alkaloids were detected, but adequate data for characterization were not obtained. Previously unreported alkaloids are designated by asterisks and will be reported in detail elsewhere.

^b Two isomers occur: the first to elute (**223Haa**) is the exo, exo (5Z, 8E) isomer identical with a thief ant pyrrolizidine (Jones et al., 1980).

^c The alkaloid designated **223A**, here listed as a 5,8-disubstituted indolizidine, may represent more than one alkaloid and even more than one structural class. Initially, **223A** was postulated to be quinolizidine (Daly et al., 1992). But further analyses indicate that **223A** in many extracts is an 8-ethyl-5-propylindolizidine with, in addition, a 6-ethyl substituent (unpublished results).

^d “Tricyclics” refer to compounds whose mass spectra appear related to those of the coccinellines, which occur in beetles (Ayer et al., 1976).

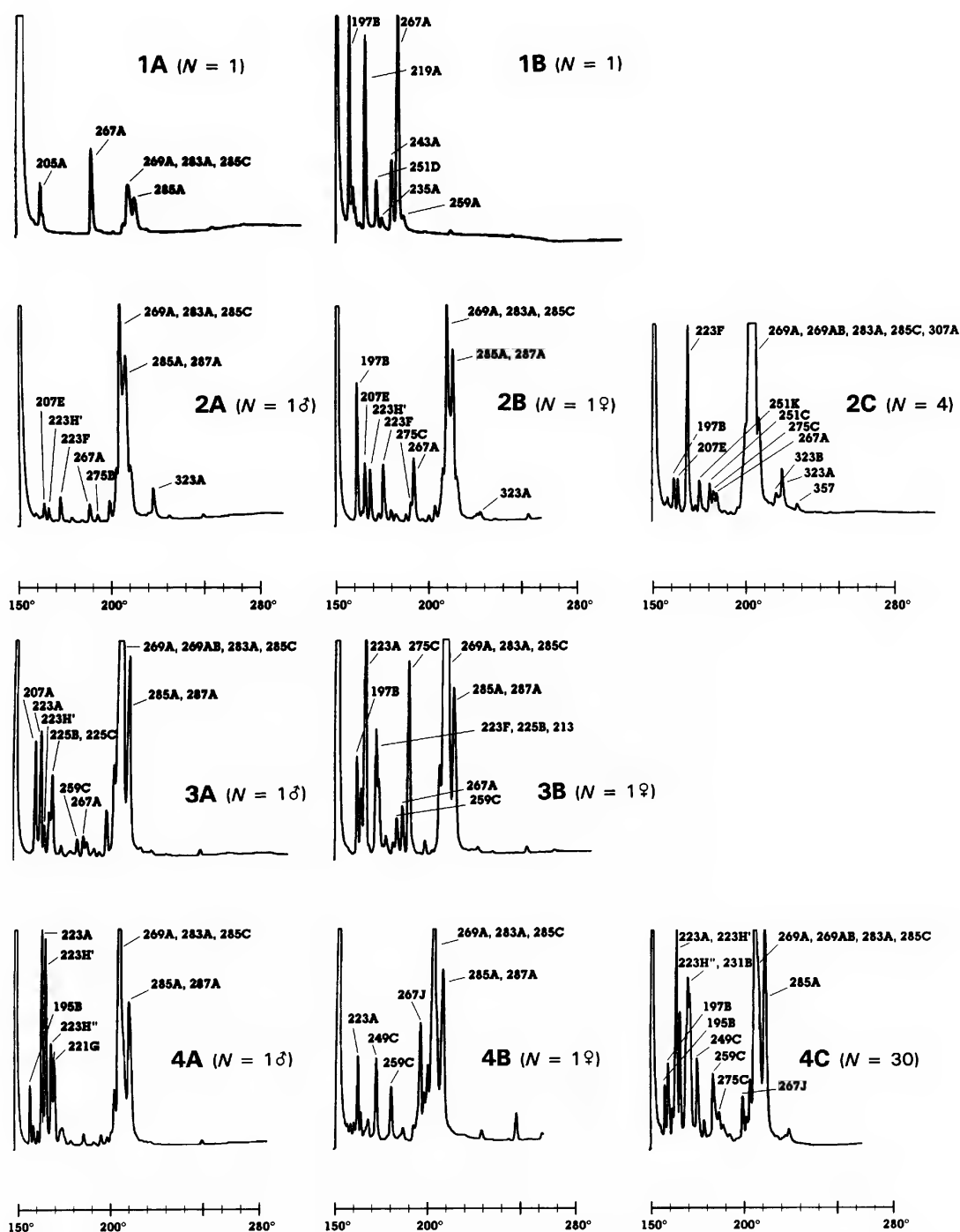


Fig. 5. Gas chromatographic traces showing variation in alkaloid profiles from individual skins and two pooled samples of *Dendrobates granuliferus* (1–3) and a sympatric population of *D. pumilio* (4), all from southern Costa Rica, as follows:

Pacific versant: *D. granuliferus*, 1. Near Puerto Jiménez, Península de Osa, Puntarenas Province, Jan. 1987. 2. Río Grande de Térraba, Palmar Norte, Puntarenas Prov., June 1990 (individual skins) and June 1992 (4 pooled skins). **Atlantic versant:** 3. *D. granuliferus* and 4. *D. pumilio* in sympatry at Río Sand Box, near Bribri, Limón Prov., June 1990.

brightly colored dendrobatid frogs. The great majority of these compounds were previously unknown in nature and many represented new classes of compounds based on novel chemical structures. Many also proved to have marked effects on nerve and muscle and several rapidly became important molecular probes in neurophysiological research. The most distinctive classes of these compounds have become known as the "dendrobatid alkaloids"¹⁰ and a large body of literature has proliferated in the biomedical, chemical, and natural sciences. The phylogenetic component of this literature—the intertwined biochemical and systematic aspect—can be traced in a handful of papers, chronologically including the following: Daly and Myers (1967), Myers and Daly (1976), Daly et al. (1978), Myers et al. (1978), Daly et al. (1987), and Myers et al. (1991).

Systematic implications of the alkaloid data have been viewed cautiously from the beginning owing to problems inherent in the use of data involving micromolecular, secondary metabolites (e.g., discussion by Myers and Daly, 1976: 194–197). But, despite problems of detection and homology, it was believed that all the dendrobatid alkaloids were synthesized by the frog that contained them and that identical alkaloids in different species of frogs were supportive of a common evolutionary event. This belief was based on the novelty of many of the frog alkaloids and on a failure to detect alkaloids or potential precursors in extracts of stomach contents (unpubl. data). Not explained, however, were observations that wild-caught frogs showed marked declines in toxicity and captive-reared ones were nontoxic (e.g., Myers et al., 1978: 336; Daly et al., 1980).

¹⁰ Some of these compounds are now also known from other small, usually brightly colored frogs and toads in the southern hemisphere (*Mantella*, *Melanophryniscus*, *Pseudophryne*. See Daly et al. 1984, 1990, 1993; Garraffo et al., 1993a, 1993b).

But now, based on experiments with captive-raised frogs (mostly *Dendrobates auratus*, but some experiments with *Epipedobates tricolor* and *Phyllobates bicolor*), it appears that at least some of the dendrobatid alkaloids are the result of dietary uptake and sequestering (Daly et al., 1992, 1993, 1994b, 1994c).

Alkaloids of the following classes were found to accumulate in the skin when added as dietary supplements (Daly et al., 1994b):

Histrionicotoxins
Decahydroquinolines
3,5-Disubstituted Indolizidines
1,4-Disubstituted Quinolizidines
Pumiliotoxin and Allopumiliotoxins (trace accumulations only)

On the other hand, 2,5-disubstituted pyrrolizidines and 2,6-disubstituted piperidines did not accumulate in *D. auratus*. Captive-raised *Phyllobates bicolor* accumulated very low levels of batrachotoxinin-A, but there was no conversion to batrachotoxin/homobatrachotoxin. No alkaloid accumulation was detected in species of *Colostethus*.

Alkaloids of the following classes were found to accumulate in the skin when leaf-litter arthropods (collected with Berlese funnels) were fed over a period of seven months to captive-raised *Dendrobates auratus* (Daly et al., 1994c):

Histrionicotoxins (19-carbon)
Decahydroquinolines (19-carbon)
1,4-Disubstituted Quinolizidines
Gephryrotoxins (19-carbon)
Pyrrolizidine oximes
Coccinellines and related tricyclic alkaloids

Alkaloids in skin of wild-caught *Dendrobates auratus* that were *not* found in captives raised on leaf-litter insects included pumiliotoxins, allopumiliotoxins, homopumiliotoxins, decahydroquinolines (13-, 15-, and 17-carbon), histrionicotoxins (15- and 17-carbon), and 3,5- and 5,8-disubstituted indolizidines.

←
Number and letter designations of samples match those in table 4. The gas chromatographic column is programmed from 150 to 280°C at 10°C per minute; other chromatographic conditions as given in Daly et al. (1987, fig. 1 legend). Emergent temperatures can differ somewhat with different columns and variations in flow rates.

Possible arthropod sources for some "dendrobatid" alkaloids include ants (3,5-disubstituted pyrrolizidines and 3,5-disubstituted indolizidines), beetles (coccinellines), and small millipedes (pyrrolizidine oximes). There are, however, no known arthropod sources for decahydroquinolines, gephyrotoxins, histrionicotoxins, pumiliotoxins, allopumiliotoxins, homopumiliotoxins, 5,8-disubstituted indolizidines, 1,4-disubstituted quinolizidines, epibatidines, or batrachotoxins. The origin of these many compounds therefore remains an open question.

But knowledge that at least some alkaloids result from dietary uptake may help explain many puzzling observations, including the unprecedented variation between alkaloid profiles of individual specimens of *Dendrobates granuliferus* and *D. pumilio* (this paper), and much (but probably not all) of the sometimes chaotic geographic variation in skin toxins of individual species, including neighboring populations of *Dendrobates pumilio* in western Panama (summary in Myers and Daly, 1983).¹¹

Many studies of dendrobatids and their alkaloids were based on one or more standard 10-skin samples for each population studied. Such samples were repeatable and the profiles agreed in the relative amounts of compounds in large skin samples obtained for chemical and pharmacological study (unpubl. data). Clearly, however, individual and temporal variation in some populations of *D. pumilio* and *D. granuliferus* show that the adequacy of 10-skin samples for comparative purposes can no longer be taken for granted.

Although alkaloid profiles have been routinely published as part of new species de-

scriptions, the data have rarely been found useful at the level of supporting taxonomic difference between related species. The main exception involved assignment of names to two new sympatric populations of *Dendrobates*: Based on size, habits, and skin chemistry, one new population was assigned to the wide-ranging, highly variable *D. histrionicus* and the other, *D. occultator*, was described as new (Myers and Daly, 1976: 223–225, 244). In the same paper (pp. 223–224, 240), the allopatric *D. lehmanni* was described as new, based on its mostly cross-banded color pattern and on a set of alkaloids different from all populations of *D. histrionicus*. The last is the only dendrobatid in which variation in skin alkaloids is thought to show geographic patterns, with a strong tendency toward nearest neighbor effects within a broader differentiation between southern and northern populations. This hypothesis (Myers and Daly, 1976) was corroborated in a follow-up study (Daly et al., 1986) and hardly seems explicable by presumptions of dietary variation.

Despite the continuing need for caution, none of the several taxonomic decisions based on the dendrobatid alkaloids are invalidated by the evidence of dietary uptake. Sequestering some compounds does not logically preclude the possibility that others are synthesized in the elegant granular glands as suggested by Neuwirth et al. (1979)—but even this consideration is not vital to the taxonomic system. There is ample reason to conclude that genetic mechanisms are in operation, both for dietary intake/uptake and, at least in *Phyllobates*, for an inherited insensitivity to action of the skin toxins.

Species of *Dendrobates*, *Epipedobates*, and *Phyllobates* (and presumably *Minyobates*) have efficient systems for incorporating dietary alkaloids into the skin and retaining such alkaloids for long periods, whereas non-toxic *Colostethus* appear to lack the ability to take up lipophilic alkaloids (Daly et al., 1994b).¹² Even though captive-raised *Phyl-*

¹¹ At first it might seem unlikely that the very different alkaloid profiles of neighboring *D. pumilio* populations could be the result of significantly different suites of prey items. One must remember, however, that interpopulational variation in behavior and microhabitat utilization is extraordinary in this species (Daly and Myers, 1967; Myers and Daly, 1983). Nonetheless, although the uptake system appears to allow accumulation of diverse alkaloids, the process can be selective. In the case of *D. auratus*, for example, an ant indolizidine was accumulated, whereas a pyrrolizidine from the same ant was not (Daly et al., 1994b). If, as seems likely, there is a degree of individual variability in the uptake system, then small or newly founded dendrobatid populations might come to diverge in their defensive secretions by chance alone.

¹² The only species of *Colostethus* known to be toxic contains water-soluble compounds recently identified as tetrodotoxin and related compounds, conceivably derived from external sources (Daly et al., 1994a). Such compounds have not been found in the aposematic dendrobatids.

lobates terribilis are nontoxic, they retain impunity to the very potent batrachotoxins owing to alteration of a regulatory site controlling sodium-channel activation and permeability (Daly et al., 1980). All these differences have a presumed genetic basis irrespective of the origin of the skin alkaloids. Indeed, it is likely that only frogs with impunity to batrachotoxins (*Phyllobates*) could target dietary sources containing such toxic substances.

The presence of dendrobatid alkaloids is thus correlated with genetic mechanisms, which seem to vary within the Dendrobatidae at least to the extent in which specific alkaloids are accumulated from dietary sources and/or biosynthesized. This variation has been used systematically to help sort out only the following supraspecific groupings:

1. A monophyletic group comprising the genera *Epipedobates*, *Minyobates*, *Phyllobates*, and *Dendrobates*, which are mostly brilliantly colored and which contain in total a great variety of lipophilic alkaloids, some classes of which are shared by all genera. The group collectively is known as "aposematic dendrobatids" or "tropical poison frogs." *Epipedobates* is the basal plesiomorphic assemblage; it comprises species groups whose relationships should be resolved before further generic partitioning (Myers et al., 1991: 18).
2. The genus *Minyobates* (dwarf tropical poison frogs) seems partially supported by lack (= presumed loss) of the ability to accumulate alkaloids of the histrionicotoxin class, which occurs in the other genera of aposematic dendrobatids. The decahydroquinoline class seems to have been virtually abandoned also.¹³ *Minyobates* resembles *Dendrobates* in derived but possibly homoplastic features of hand structure and lack of teeth (teeth also lacking in some species of *Epipedobates*), but *Minyobates* differs in very small size and in such primitive features as an incom-

plete oblique lateral stripe (some species), cephalic mating amplexus (at least in the type species), and in the tadpole having a laterally indented oral disc and dextral anus.

3. Monophyly of the genus *Phyllobates* (true dart-poison frogs) is supported by ontogenetic considerations of color pattern and by presence of the remarkably toxic batrachotoxins, a class of steroidal alkaloids that are lacking in other dendrobatids but which are accumulated by *Phyllobates* often to the exclusion of other alkaloids.
4. *Phyllobates* + *Dendrobates* form a monophyletic group based on shared presence of 3,5-disubstituted indolizidines and shared loss of cephalic amplexus during mating, as well as by a complete loss of the primitive oblique lateral stripe. *Dendrobates* appears to differ from other dendrobatids in that the larvae have an unindented oral disc and a median anus. Species of *Dendrobates* tend to be astonishingly variable in coloration and to have remarkable capacity for accumulation of skin toxins (most minor and all major classes of dendrobatid alkaloids except batrachotoxins), but there is no alkaloid synapomorphy uniting these species. A recent worker who used histrionicotoxins as an apomorphy of *Dendrobates*, perhaps based on Myers et al. (1978: 332–333), did not notice that these compounds are now known from various *Epipedobates* and *Phyllobates*.

In addition, it should be noted that absence of some kind of defensive compounds (not necessarily lipophilic alkaloids, see footnote 12) in the granular glands of most *Colostethus* might be due to synapomorphic loss rather than symplesiomorphic absence (Myers et al. 1991: 28–29).

CONCLUSIONS

Comparisons of calls and allozymes negate the possibility that skin texture and color differences between sympatric frogs might be due to intrapopulation polymorphism at the Rio Sand Box site. The names *Dendrobates pumilio* O. Schmidt and *Dendrobates granuliferus* Taylor are assigned to the popula-

¹³ Although this class is generally absent in *Minyobates*, decahydroquinoline 243A does occur in *M. bombetes*. The dominant alkaloids in *Minyobates* are the pumiliotoxin-A class, although 5,8-disubstituted indolizidines and 1,4-disubstituted quinolizidines also occur.

tions found in sympatry in the Atlantic lowlands of southeastern Costa Rica.

Consideration of populational and individual variation in skin toxins suggests that both environmental and genetic factors are involved and that these characters have no immediate taxonomic usefulness in assigning individual populations to either of these species. Despite marked similarity in alkaloid profiles of the sympatric samples, however, certain dissimilarities suggest the possibility of differences either in diet or in ability to uptake (or to biosynthesize) specific alkaloids. The underlying genetic mechanisms of alkaloid sequestering (and biosynthesis?) are taxonomically most useful in supporting the monophyly of a few genera and suprageneric groupings of dendrobatids.

The Atlantic-side population sample assigned to *Dendrobates granuliferus* is genetically somewhat differentiated from the one Pacific-side population of *granuliferus* that was sampled. Considering close correspondence in morphology and coloration, however, the allele variation is not great enough to repudiate the hypothesis that a single (monophyletic) species is involved and that *D. granuliferus* occurs on both coasts. Extrapolating from the situation in *D. pumilio* (Escalante et al., MS.), some degree of genetic differentiation is also to be expected among the populations of *D. granuliferus* in the Golfo Dulce region.

Whether the Atlantic coast population of *D. granuliferus* is genetically more differentiated than the Golfo Dulce populations are among themselves remains to be seen. Although the Atlantic and Pacific *granuliferus* populations conceivably may have been separated since the time of the Talamancan orogeny, a more recent break is just as plausible. Given the scenario of a once continuous distribution around the northern end of the Costa Rican highlands, climatically controlled vegetational changes might have disrupted this distribution during the Pleistocene. The latter scenario makes present-day Atlantic and Pacific populations of *D. granuliferus* southern relicts of a formerly broader distribution. But, if this were so, why has *granuliferus* not been found at localities throughout the Atlantic lowlands of Costa Rica, where a rain-forest climate is main-

tained owing to orographic interruption of the northeast trades? Are we today seeing the final stage whereby *granuliferus* is being replaced by *pumilio* on the Atlantic coast? It is of interest that the vocalizations of Costa Rican *granuliferus* and Panamanian *pumilio* have a similar region of maximum energy output (peak emphasized frequency > 4000 Hz), whereas examined spectrograms of Costa Rican *pumilio* show a lower frequency with a tendency for emphasized harmonics within the range 3000–4000 Hz. Although the chirp calls of *pumilio* and those of *granuliferus* differ in several respects, it would not be unexpected for these similar-sized frogs to have tuned to different calling frequencies when in sympatry.

The notion that *Dendrobates granuliferus* and *D. pumilio* are each other's closest relatives was not based on synapomorphy but rather on logical supposition and a previous unawareness that the *D. histrionicus* group is fairly speciose in Costa Rica and the western half of Panama (Myers et al., 1984; Myers, unpubl.). In defining the *histrionicus* group as a monophyletic unit, Myers et al. (1984: 19) pointed out that its geographic distribution paralleled that of *Phyllobates*, with which it may have shared a similar history (see also Maxson and Myers, 1985). Lahanas (1991) corroborated these congruent vicariant patterns by use of allozyme data, but his sample of the Central American section of the *histrionicus* group was limited to *D. granuliferus* and *D. pumilio*, which of necessity came out as sister species. Finding these species together at the same locality does not disprove such a close relationship, but, assuming allopatric speciation, a dispersal event would have to be hypothesized to explain their sympatric occurrence. But there seems no point in such speculation unless the degree of relatedness can be resolved.

NOMENCLATURE NOTE

Duellman (1993: 60) recently corrected the name *Dendrobates granuliferus* to *Dendrobates "granulifer."* He gave Myers (1987) as "authority" for the change. This attribution of authority is in error—Myers has never advocated such a change and did not mention the species in the paper cited.

Duellman's change presumably is based on a reasonable supposition that the second part of the compound word *granuliferus* was derived from the Latin *-fer* (from *fero*, to bear), a suffix conjugated as *-fer*, *-fera*, *-ferum*. *Dendrobates* is masculine, hence *granulifer* would be the expected derivation.

But Edward Taylor (died 1978) was not known for always doing the expected. He might, for example, have considered *-ferus* as the New Latin equivalent of *-fer*. Or he might have derived the name from Latin as follows: granule (from *granum*, a grain or seed) + connecting *i* + the adjective *ferus* (wild, but also with the connotation of "rough"). This last derivation (loosely pertaining to a state of being "roughly granular") certainly would be less elegant than *granulifer* but it would be nomenclaturally acceptable nonetheless.

Taylor (1958) did not provide an etymology when he coined *granuliferus*, the derivation of which is not unambiguous. Under the circumstances, the original spelling *granuliferus* seemingly must be preserved inas-

much as it is not "demonstrably incorrect" (1985 *Internatl. Code Zool. Nomencl.*, art. 32b).

ACKNOWLEDGMENTS

Collecting was done under permit from the Servicio de Fauna Silvestre of Costa Rica. We are especially indebted to Dr. Patricia Escalante and Ms. Margaret G. Arnold for running allozyme electrophoresis of the sympatric *Dendrobates pumilio* and *D. granuliferus* samples, and to Dr. George F. Barrowclough for providing laboratory facilities and advice. Recording equipment used at the National Aquarium in Baltimore was provided by Donald C. Forester and photographic assistance was provided by George H. Grall. Dr. E. N. Arnold kindly searched for data on the British Museum specimen of *D. granuliferus* from Sixaola. For reading and commenting on various parts of the manuscript, we thank Drs. George F. Barrowclough, Maureen A. Donnelly, Thomas F. Spande, and Richard G. Zweifel.

REFERENCES

- Allen, Paul H.
1956. The rain forests of Golfo Dulce. Gainesville: Univ. Florida Press, xi + 417 pp.
- Ayer, William A., Michael J. Bennett, Lois M. Browne, and James T. Purdham
1976. Defensive substances of *Coccinella transversoguttata* and *Hippodamia caseyi*, ladybugs indigenous to western Canada. *Can. J. Chem.* 54: 1807-1813.
- Daly, John W., and Charles W. Myers
1967. Toxicity of Panamanian poison frogs (*Dendrobates*): some biological and chemical aspects. *Science* 156: 970-973 + cover photo.
- Daly, John W., George B. Brown, Monica Mensah-Dwumah, and Charles W. Myers
1978. Classification of skin alkaloids from Neotropical poison-dart frogs (Dendrobatidae). *Toxicon* 16: 163-188.
- Daly, John W., Charles W. Myers, Jordan E. Warnick, and Edson X. Albuquerque
1980. Levels of batrachotoxin and lack of sensitivity to its action in poison-dart frogs (*Phylllobates*). *Science* 208: 1383-1385.
- Daly, John W., Robert J. Highet, and Charles W. Myers
1984. Occurrence of skin alkaloids in non-dendrobatid frogs from Brazil (Bufonidae), Australia (Myobatrachidae) and Madagascar (Mantellinae). *Toxicon* 22(6): 905-919.
- Daly, John W., Thomas F. Spande, Noel Whittaker, Robert J. Highet, D. Feigl, N. Nishimori, Takashi Tokuyama, and Charles W. Myers
1986. Alkaloids from dendrobatid frogs: structures of two ω -hydroxy congeners of 3-butyl-5-propylindolizidine and occurrence of 2,5-disubstituted pyrrolidines and a 2,6-disubstituted piperidine. *J. Nat. Prod.* 49(2): 265-280.
- Daly, John W., Charles W. Myers, and Noel Whittaker
1987. Further classification of skin alkaloids from Neotropical poison frogs (Dendrobatidae), with a general survey of toxic/noxious substances in the Amphibia. *Toxicon* 25: 1023-1095.
- Daly, John W., H. Martin Garraffo, L. K. Pannell, Thomas F. Spande, C. Severini, and V. Ersparmer
1990. Alkaloids from Australian frogs (Myobatrachidae): pseudophrynamines and pumiliotoxins. *J. Nat. Prod.* 53(2): 407-421.
- Daly, John W., Sherrie I. Secunda, H. Martin Garraffo, Thomas F. Spande, Anthony Wisnieski,

- Charles Nishihira, and John F. Cover, Jr.
1992. Variability in alkaloid profiles in Neotropical poison frogs (Dendrobatidae): genetic versus environmental determinants. *Toxicon* 30: 887–898.
- Daly, John W., H. Martin Garraffo, and Thomas F. Spande
1993. Amphibian alkaloids. In G. A. Cordell (ed.), *The Alkaloids*, vol. 43, pp. 185–288. San Diego, Calif.: Academic Press.
- Daly, John W., Fabian Gusovsky, Charles W. Myers, Mari Yotsu-Yamashita, and Takeshi Yasumoto
1994a. First occurrence of tetrodotoxin in a dendrobatid frog (*Colostethus inguinialis*), with further reports for the bufonid genus *Atelopus*. *Toxicon* 32(2): 279–285.
- Daly, John W., Sherrie I. Secunda, H. Martin Garraffo, Thomas F. Spande, Anthony Wisnieski, and John F. Cover, Jr.
1994b. An uptake system for dietary alkaloids in poison frogs (Dendrobatidae). *Toxicon* 32(6): 657–663.
- Daly, John W., H. Martin Garraffo, César Jaramillo, and A. Stanley Rand
1994c. Dietary source for skin alkaloids of poison frogs (Dendrobatidae)? *J. Chem. Ecol.* 20(4): 943–955.
- Duellman, William E.
1993. Amphibian species of the world: additions and corrections. *Univ. Kansas Mus. Nat. Hist.*, special publ. 21: iv + 372 pp.
- Escalante, Patricia, Margaret G. Arnold, and Charles W. Myers
MS. Explosive polymorphism and phylogeny in a group of tropical poison frogs (*Dendrobates pumilio* and relatives): results from allozyme electrophoresis. Manuscript in progress.
- Garraffo, H. Martin, Thomas F. Spande, John W. Daly, A. Baldessari, and E. G. Gros
1993a. Alkaloids from bufonid toads (*Melanophryniscus*): decahydroquinolines, pumiliotoxins and homopumiliotoxins, indolizidines, pyrrolizidines, and quinolizidines. *J. Nat. Prod.* 56(3): 357–373.
- Garraffo, H. Martin, J. Caceres, John W. Daly, Thomas F. Spande, N. R. Andriamaharavo, and M. Andriantsiferana
1993b. Alkaloids in Madagascan frogs (*Mantella*): pumiliotoxins, indolizidines, quinolizidines, and pyrrolizidines. *J. Nat. Prod.* 56(7): 1016–1038.
- Jones, Tappey H., Murray S. Blum, Henry M. Fales, and Catharine R. Thompson
1980. (5Z,8E)-3-Heptyl-5-methylpyrrolizidine from a thief ant. *J. Org. Chem.* 45: 4778–4780.
- Jones, Tappey H., Murray S. Blum, and Henry M. Fales
1982. Ant venom alkaloids from *Solenopsis* and *Monomorium* species: recent developments. *Tetrahedron* 38: 1949–1958.
- Jungfer, Karl-Heinz
1985. Beitrag zur Kenntnis von *Dendrobates speciosus* O. Schmidt, 1857 (Salientia: Dendrobatidae). *Salamandra* 21(4): 263–280.
- Lahanas, Pete N.
1991. Phylogenetic relationships among the dendrobatid frogs *Phyllobates* and *Dendrobates*: preliminary electrophoretic evidence for congruent vicariance patterns in Central and South America. Joint ann. meet. Soc. Study Amphib. Rept. and Herpetol. League, Pennsylvania State Univ., Aug. 1991, Program and abstracts, p. 75.
- Maxson, Linda R., and Charles W. Myers
1985. Albumin evolution in tropical poison frogs (Dendrobatidae): a preliminary report. *Biotropica* 17(1): 50–56.
- Meyer, Eberhard
1993. Fortpflanzung und Brutpflegeverhalten von *Dendrobates granuliferus* Taylor, 1958 aus Costa Rica (Amphibia: Anura: Dendrobatidae). *Veröff. Naturhist. Mus. Schleusingen* 7/8: 113–142.
- Myers, Charles W.
1987. New generic names for some Neotropical poison frogs (Dendrobatidae). *Pap. Avulsos Zool. (São Paulo)* 36(25): 301–306.
- Myers, Charles W., and John W. Daly
1976. Preliminary evaluation of skin toxins and vocalizations in taxonomic and evolutionary studies of poison-dart frogs (Dendrobatidae). *Bull. Am. Mus. Nat. Hist.* 157(3): 173–262 + color pls. 1–2.
1983. Dart-poison frogs. *Sci. Am.* 248(2): 120–133.
- Myers, Charles W., John W. Daly, and Borys Malkin
1978. A dangerously toxic new frog (*Phyllobates*) used by Emberá Indians of western Colombia, with discussion of blowgun fabrication and dart poisoning. *Bull. Am. Mus. Nat. Hist.* 161(2): 307–366 + color pls. 1–2.
- Myers, Charles W., John W. Daly, and Víctor Martínez
1984. An arboreal poison frog (*Dendrobates*)

- from western Panama. *Am. Mus. Novitates* 2783: 20 pp.
- Myers, Charles W., Alfredo Paolillo O., and John W. Daly
1991. Discovery of a defensively malodorous and nocturnal frog in the family Dendrobatidae: phylogenetic significance of a new genus and species from the Venezuelan Andes. *Am. Mus. Novitates* 3002: 33 pp.
- Nei, Masatoshi
1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics* 89: 583–590.
- Neuwirth, Maria, John W. Daly, Charles W. Myers, and Lois W. Tice
1979. Morphology of the granular secretory glands in skin of poison-dart frogs (Dendrobatidae). *Tissue & Cell* 11(4): 755–771.
- Savage, Jay M.
1968. The dendrobatid frogs of Central America. *Copeia* 1968(4): 745–776.
1982. The enigma of the Central American herpetofauna: dispersals or vicariance? *Ann. Missouri Bot. Garden* 69: 474–547.
- Silverstone, Philip A.
1975. A revision of the poison-arrow frogs of the genus *Dendrobates* Wagler. *Nat. Hist. Mus. Los Angeles County, Sci. Bull.* 21: vi + 55 pp.
- Swofford, David L., and Richard B. Selander
1989. Biosys-1. A computer program for the analysis of allelic variation in population genetics and biochemical systematics. Release 1.7. Illinois Natural History Survey.
- Taylor, Edward H.
1958. Additions to the known herpetological fauna of Costa Rica with comments on other species. No. III. *Univ. Kansas Sci. Bull.* 39(1): 3–40.
- Wright, Sewall
1978. Evolution and the genetics of populations, vol. 4. Variability in and among natural populations. Univ. Chicago Press, x + 580 pp.

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